Probing active forces via a fluctuation-dissipation relation: Application to living cells

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Probing active forces via a fluctuation–dissipation relation: application to living cells

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Abstract. - We derive a new fluctuation–dissipation relation for non–equilibrium systems with long term memory. We show how this relation allows one to access new experimental information regarding active forces in living cells that cannot otherwise be accessed. For a silica bead attached to the wall of a living cell, we identify a cross-over time between thermally controlled fluctuations and those produced by the active forces. We show that the probe position is eventually slaved to the underlying random drive produced by the so-called active forces.

Living cells are paradigmatic out of equilibrium systems, subjected to the ATP-driven activity of a collection of molecular motors, whose individual motion cannot easily be disentangled from thermal fluctuations. These are relatively small systems for which fluctuation phenomena are prominent, as investigated in recent works [1–9], with specific focus on the active forces and non-Newtonian rheology [1–3, 10] which can be measured via micro-rheological devices. Our goal in the present work is to show how the cell body's random pull-and-push can be investigated by means of very recent theoretical advances in the field of non–equilibrium statistical mechanics. And indeed, from the theory standpoint, in the recent past, much effort has been invested in deriving simple generalizations or extensions of the celebrated fluctuation–dissipation theorem for systems that can be arbitrarily far out of equilibrium [5, 11–23]. These efforts have given birth to a flurry of formulas relating the response of a system to a small external perturbation to some correlation functions, even when the system is not in an equilibrium state. Yet, so far none of these formulas has been of any predictive power in an actual experimental system. Existing experiments revolving around these theoretical advances have been confined to refined and nontrivial confirmations that in some small scale systems such as an optically trapped Brownian particle [24–26] the various ingredients entering these extended fluctuation-dissipation relations (EFDR) can indeed be measured. Given that the dynamics of living cells exhibit strong memory effects, we will first have to derive our own version of an EFDR adapted to a system with stochastic yet non-Markovian dynamics. The latter EFDR and its consequence for the understanding of active forces are the central topics of this letter. For instance, how relevant is the long term memory in relating response and fluctuations? What new pieces of information on non–equilibrium forces can be learnt from such a relationship? Can thermal fluctuations be disentangled from those arising from the active forces in a quantitative way? These are the questions we wish to address in the present work.

Our experimental system, which has already been considered in [6] for other purposes, consists of a pre–muscular (C2C12) cell to which we slave a micron sized silica probe specifically bound to the membrane (in [2] the authors used a human airway smooth muscle cell). Its dynamics strongly feels the visco–elastic non-Newtonian underlying medium [2], which gives rise to memory (non-Markovian) effects. A schematic view of our experimental setup is shown in FIG. 1. According to [1, 27–30], we consider that linear-viscoelasticity correctly describes the mechanical cell behavior. The bead thus evolves under the combined effect of thermal fluctuations $\xi(t)$ and active forces $F_a(t)$, which represent all the non-thermal stochastic effects due to cellular activity. Discarding inertial effects, the position x of the bead is governed by the Langevin equation

$$\int_{-\infty}^{t} dt'\, \gamma(t-t') \frac{dx(t')}{dt'} = F_a(t) + \xi(t)\ , \qquad (1)$$

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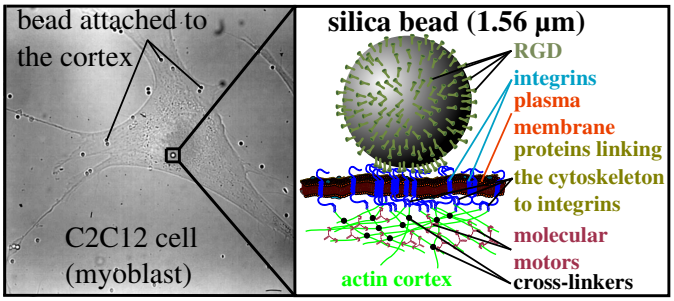

Fig. 1: Schematic view of the bead probe as attached to the cytoskeleton. The active forces are the by-product of the ATP-driven molecular motors activity.

where ξ a Gaussian colored noise with correlations $\sigma(\tau) = \langle \xi(t)\xi(t+\tau)\rangle$. The system is thermalized at an inverse temperature $\beta = 1/k_{\text{B}}T$, and we assume that active forces will not affect the thermal fluctuations, which then verify a local equilibrium condition $\sigma(\tau) = \gamma(|\tau|)/\beta$. This assumption is discussed in [31] and relies on a time-scale separation which is experimentally observed in our system, as we shall show later. The active forces are modeled by an isotropic stationary random force $F_a(t)$. While the presence of the memory kernel $\gamma(\tau)$ looks like an innocuous extension of the well-known overdamped Langevin equation (for which $\gamma(\tau)$ is to be replaced by a Dirac delta in time), we shall soon see that a number of complications emerge.

We begin with presenting the new theoretical result we wish to exploit, namely a relationship between three quantities, two of which are accessible to measurement. Let $R(t)$ be the impulse response function of the bead position at time t. Let also $\Delta x^2(t) = \langle (x(t) - x(0))^2 \rangle$ be the mean square displacement of the bead's position. Our goal is to connect R and Δx^2 to the statistics of the active force F_a.

We start by considering the effect on the bead of an applied force $f_{exp}[x(t), t]$, which explicitly depends on time t and possibly on position x. The impulse response of the bead measures the effect of the applied force on the average position, and is defined by the convolution $\delta\langle x\rangle(t) = \int dt' R(t - t') \langle f_{exp}[x(t'), t']\rangle$, where $\delta \langle x\rangle$ is the change in average position caused by the applied force. In a viscoelastic medium this is often described by means of the creep function [32], which is simply the derivative of our response function [33]. At equilibrium the Fluctuation–Dissipation Theorem relates the response to the mean square displacement $\Delta x^2(t)$. By generalizing this result, it can be shown that an extra correlation function $\langle F_a(t')x(t)\rangle$ is involved (technical details are given in [34]). Since we are dealing with a non–stationary system which exhibits anomalous diffusion, we shall only consider the time–translation invariant part of the correlation function, which is equivalent to look at the function $\langle F_a(0)x(t)\rangle$. Its expression is given by [34]:

$$\langle F_a(0)x(t)\rangle = \frac{\beta}{2}\int_{-\infty}^{t} dt'\sigma(t-t')\frac{d}{dt'}\Delta x^2(t') - \int dt' R(t')\sigma(t-t') \ . \quad (2)$$

It is worth noting that the last term above corresponds exactly to the thermal noise–position correlation function $\langle \xi(0)x(t)\rangle$. When there is no active force, (2) yields to the standard result:

$$R(t) = \frac{\beta}{2}\frac{d}{dt}\Delta x^2(t) \ , \quad \text{for } t > 0. \quad (3)$$

The originality of our approach is summarized in formula (2). This formula expresses the cross-correlation between two observables, namely the force exerted on the bead and its position, while most of the published work in the field focuses on the autocorrelation function of a single observable (force or position). Indeed, several recent works attempt to relate the force autocorrelation function to the mean square displacement of the probe [2, 6, 8, 35]. Alternatively, the calculation derived here provides an explicit expression for $\langle F_a(0)x(t)\rangle$, and makes this quantity easily computable from experiment, provided that one is able to measure the mean square displacement of the bead and its impulse response function, as shown below.

We shall now apply our result to probe active forces in living cells. The experimental set-up is the same as exposed in [6] and consists of a small bead attached through RGD-integrin links to the cortex of a pre-muscular cell (see FIG.1). First, we tracked the free diffusion of the bead on the cell cortex at room temperature ($\sim$ 298K). This allowed us to measure the mean square displacement of the probe as a function of time $\langle \Delta x^2(t)\rangle$, shown in FIG. 2. Possible mechanical drifts obtained from the motion of a bead attached to the glass substrate have been subtracted for each experiment. The mean-square displacement exhibits a two-step growth characterized by an early sub-diffusion, up to a 1s timescale yet to be interpreted, followed by super-diffusion also observed in [36]. The 1.5 exponent measured from FIG. 2 in the super-diffusive regime may vary in the range 1.3-1.7, according to the density of RGD ligand at the bead surface. Thus its value has no universal character, but it is close to the 3/2 prediction one would get assuming a linear decay for the overall noise terms (i.e. thermal–noise plus active force correlation function) [1]. It is also worth to note that we do not see any saturation effects to the mean square displacement, as the area covered by the bead after 25s is about only 1% of the projected bead surface. This corroborates our modelling in terms of a generalized Langevin equation (1) for a free particle. Of course at larger time scales it is possible that some caging effect from the actin filaments and microtubules gives rise to an effective confining potential. The effect of this potential on the mean square displacement would be a saturation towards a plateau value at large time. We also acknowledge that even though what we observe is in fair agreement with the usual "power law fluid" behavior [29], the slow initial subdiffusion could be interpreted as a confinement towards which the bead has not yet relaxed. Second, we

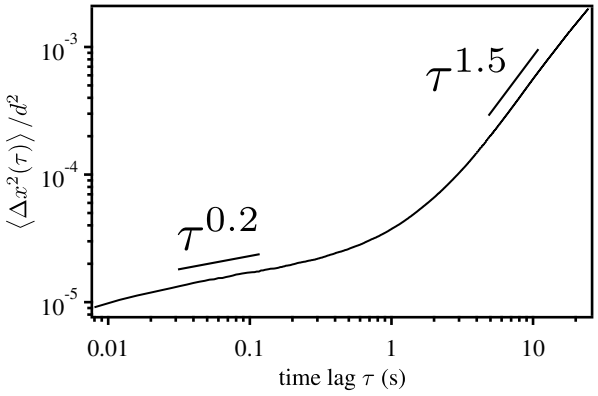

Fig. 2: The probe mean square displacement as a function of time when there is no external applied force. A definite crossover time is found at $\tau \simeq 1$ s.

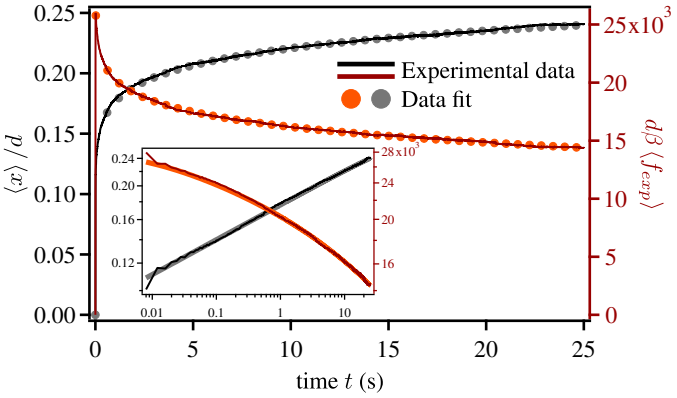

Fig. 3: Average particle position (black) and applied force (red) in dimensionless units (d is the particle diameter), as a function of time. Inset shows the same plot on a Log-Log scale. Data are very accurately interpolated by a sum of two power laws.

measured the response function by applying optical tweezers to the bead. The tweezers acted on the bead as a harmonic potential of spring constant $k_{opt} = 120\text{pN}/\mu\text{m}$. After switching our tweezers on we quickly translated them by $x_0 = 0.6\mu$m at time $t = 0$, creating a step force on the bead attached to the cell, and subsequent deformation of the latter. In the experiment the external force we applied is hence well approximated by $f_{exp}[x(t), t] = -k_{opt}(x(t) - x_0)\theta(t)$, assuming the displacement at $t = 0$ to be instantaneous. The distance between the bead and the center of the tweezers allowed us to measure the force applied on the bead. By repeating this experiment we measured the average displacement and the average force shown in FIG. 3. The force suddenly increases within a single time step, i.e. on a time lapse less than 0.004s. Then, it slowly decreases towards 0, while the bead displacement increases following a power law behavior (except in the initial steps of the relaxation), as shown in the Log-Log scale inset. This is compatible with the visco-elastic rheology responsible for the sub-diffusivity of the mean square displacement [8,37,38]. Although a single power law accurately represents the data in the figure, we chose to fit them by a sum of two power laws, in order to get the best analytic interpolation required for subsequent analysis.

All the experimental data are discretized in time. We first measure trajectories of the bead position when no external force is applied. We have collected $N_s^0 = 39$ independent sample trajectories of 250s each with a time step of 0.004s (each trajectory consists of $N_p^0 = 62500$ points). This yields a set of vectors x_i^j, where the subscript i stands for the time position and superscript j refers to the trajectory number. We compute the mean square displacement as $\langle \Delta x^2 \rangle_i = \sum_{j,k} (x_j^k - x_{j+i}^k)^2 / N_s^0 (N_p^0 - i)$, from which we take the discrete time derivative needed in (2). A second set of measurements is taken when we apply the external force on the bead. Then we measure both the position $\langle x \rangle$ and the external force $\langle f_{exp} \rangle$, as shown in FIG. 3. All curves are obtained over a time window of 25s with a time step of $\Delta t = 0.004$s and ensemble averages are taken over 39 realizations. Our goal is to recover an estimate of $\langle F_a(0)x(t) \rangle$ from (2) by discretizing integrals and derivatives. To obtain the discrete response function R_i we had to deconvolve the discrete external force $\langle f_{exp} \rangle$ from $\langle x \rangle$. For discrete data this reduces to a matrix inversion, as $\langle x \rangle_i = \sum_j \Delta t \langle f_{exp} \rangle_{ij} R_j$, where $\langle f_{exp} \rangle_{ij}$ is the triangular matrix with elements $\langle f_{exp} \rangle((i-j)\Delta t)$. This procedure is generally sensitive to noise amplification. In order to avoid this phenomenon we have used *ad hoc* numerical fits of the experimental data. Although the experimental data is fairly well fitted by a single power law, we have preferred to use a sum of two power laws, which is more precise at short time. The fit accuracy can be seen in FIG. 3, where we compare experimental data with data fit. Likewise, we measured $\gamma_i \equiv \gamma(i\Delta t)$ by inverting the equation $\langle f_{exp} \rangle_i = \sum_j \left[\frac{d\langle x \rangle}{dt} \right]_{ij} \gamma_j$. We then computed σ_i as $1/\beta(\gamma_i + \gamma_{-i})$.

With the knowledge of σ, $\Delta x^2(t)$ and R it is then possible to explicitly compute the cross correlation function (2). We did this by computing integrals as Riemann sums with step size Δt and derivatives as forward finite differences. The result is shown in FIG. 4, where we have plotted the correlation functions $\langle F_a(0)x(t) \rangle$ and $\langle \xi(0)x(t) \rangle$. As expected, the thermal noise–position correlation function is of the order of $k_B T$ and decreases to $k_B T$ after a time of the order of the second. For comparison, simpler systems with memory decaying exponentially fast, exhibit for this function a growth from 0 to $2k_B T$. Besides, the active force–position correlation function has a completely different behavior. First, we observe that the short time behavior exhibits small yet significant negative correlation. This would mean that on average the particle moves *opposite* to the active force for about the first half second. At later times, as expected the correlation function turns positive and grows linearly with time, which implies that the particle on average moves in the same direction of the active forces. However we can now quantify this correlation, which turns out to be of $10 - 100 k_B T$. Of course we expect that at later times the correlation function should saturate to a limiting value. Unfortunately so far our experimentally accessible time window

does not allow to explore this regime (at which one would also see saturation of the mean square displacement, as discussed earlier). The figure also shows that for short time ($< 1s$) the effect of thermal noise prevails, validating our local equilibrium assumption discussed earlier. At large times the active force dominates, and the crossover timescale between these two regimes is of the order of 1s. This seems to corroborate a scenario for which the short time behavior has equilibrium–like properties, from which the sub-diffusive nature of visco–elasticity emerges, while the long time behavior is strongly governed by active non–equilibrium forces. Finally we briefly comment on the initial anti–correlation exhibited by the active force–position correlation function. This feature appears for the first half second of the measurements. Even though in this time range the data fit (as shown in FIG. 3) is less accurate than at later times, the anti-correlation seems to be quite robust [1]. So far we do not have a clear interpretation for this phenomenon. However, we can get some hints from the following scenario. As we have seen, the short time dynamics could be seen as an equilibrium–like diffusion in a localized potential determined by the local structure of the cell cortex. Then, the effect of the active forces at these timescales could generate a displacement of this potential. We have checked that this simple model (where we are neglecting memory effects) is able to exhibit such a negative correlation. We cannot however yet reproduce quantitatively our observed results within this simple toy model, whose refinements are left for future work. Another useful information that can be extracted from the correlation function is concerned with the power spent by the active forces on the bead. This can be estimated from the time derivative $d/dt \langle F_a(0)x(t)\rangle$ when $t \to 0$. Our experimental data shows that this power is about $30 k_B T/s$. Assuming a ballistic–like behavior, we estimate from FIG. 2 a typical velocity of $v \sim 5 \times 10^{-3}$ μm/s (compatible with 0.08μm/s observed in [39]). This leads to an applied force of almost 25 pN, which would mean that a small number (five or less) of motors are contributing to the bead motion.

The equilibrium fluctuation–dissipation theorem relates the response of the system to a small perturbation to spontaneous fluctuations in equilibrium. Out-of-equilibrium extensions involve, besides dissipative aspects, also kinetic aspects where the dynamical activity appears within an extra correlation function with the physical observable under scrutiny. For overdamped Langevin dynamics, and even for dynamics with strong memory effects, the latter is directly related to the non–equilibrium forces driving the system out of equilibrium. Understanding the properties of these forces in the dynamics of living cells is a problem of its own. With this new theoretical tool we have shown how to access some of the properties of these forces. We have been able to quantify the time scale at which active forces driven fluctuations win over thermal ones. Another quantitative spin-off is an estimate for the power dissipated by the active forces into the system, which turns out to be three orders of magnitude smaller than the chemical power

[1] We checked that by considering overestimations and underestimations of the fitted data the anti-correlation is always present.

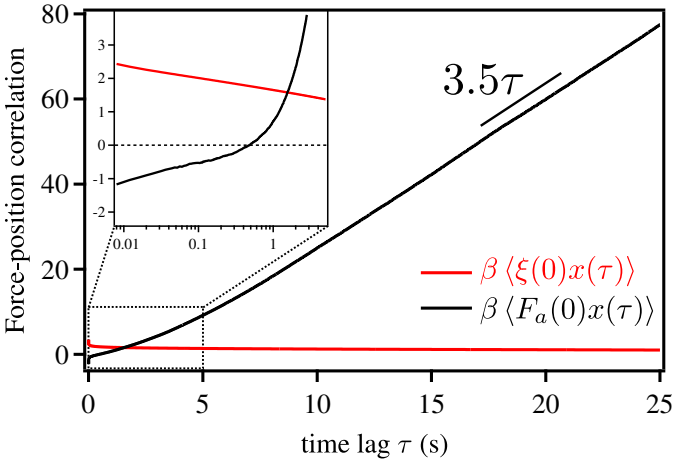

Fig. 4: Force–position correlation as a function of time lag.

injected into the underlying motors by the ATPase steps. Another conclusion that we have arrived at is the strong slaving of the probe motion to the active forces, which are coherently followed. We have thus demonstrated that extracting useful information is indeed possible, and this paves the way to a number of improvements or generalizations. An immediate modification of the experimental setup would involve the use of an optical trap to render the unperturbed state stationary, and then pulling on the particle by displacing the trap. Making the system stationary would eliminate time drifts and would simplify the subsequent theoretical analysis by allowing to focus solely on active forces. Other simple improvements of the present work would, for instance, entail tracking higher moments of the bead positions. This could be done by attempting to vary, in the latter stationary setup, the trap's stiffness, instead of the position of its minimum. Even if the rheological meaning of the response function would then be lost, other relevant information concerning fluctuations would then be gained. We leave these projects for future work.

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